CDR1 CDR2 CDR3

AR-1 AR-2 AR-3 AR-4
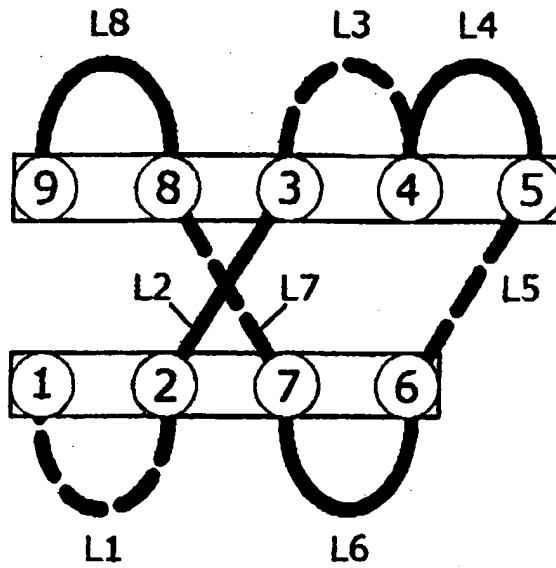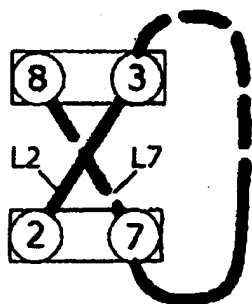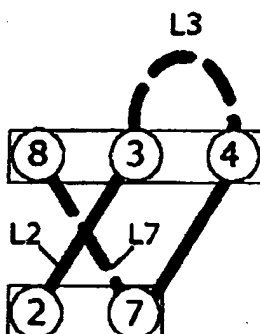
L2 L4 L6 L8

NH2

1A

1B

2 3 4 5 6 7 8

9A

9B

COOH

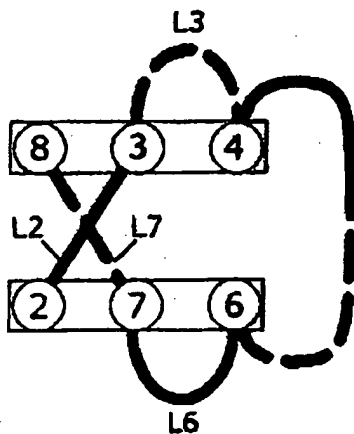L1 L3 L5 L7

Fig. 1

3D structure topology

Fig. 2

Structural Deviations

4 beta elements:

5 beta elements:

6 beta elements-a:

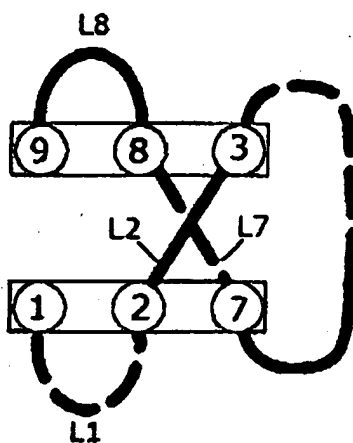**6 beta elements-b:**

1GOY: Interleukin-1
receptor type 1

1J88: Fc epsilon receptor
type alpha

Fig. 3a

7 beta elements-a:

2DLI: Immunoglobulin killer
receptor 2dl2

7 beta elements-b:

1FF5: E-cadherin domain

8 beta elements:

1IAR: Interleukin-4 alpha receptor
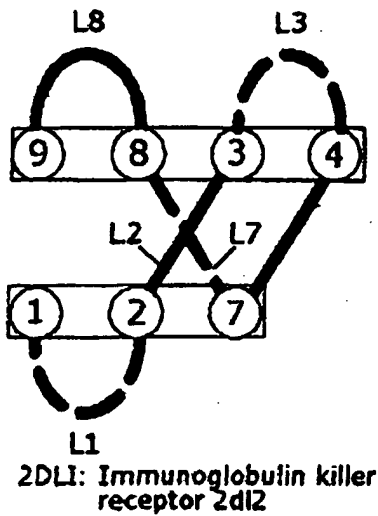
9 beta elements:
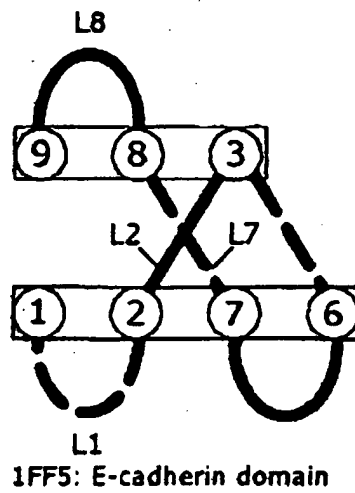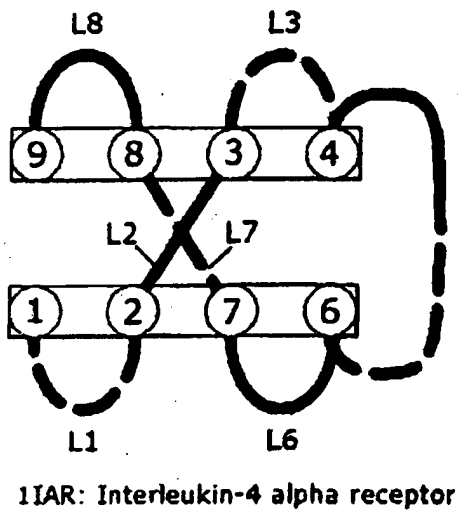
All antibody and T-cell receptor
variable domains

Fig. 3b

Modular Affinity & Scaffold Transfer (MAST) Te

Phage Display
selected Scaffold
including unique
Affinity regions

Molecular isolation of
Affinity region(s)

Target structure
for Affinity Transfer

Molecular isolation of
target framework

Hy

Fig. 4

STRUCTURAL ALIGNMENT: EXAMPLES OF 1F2X V$_{HH}$ CAMELID ANTIBODY VARIABLE FRAGMENT

```
              1              2                    3              4              5                  6                7                    8                         9
1F2X  QVQLVE-SG---G-GSVQAGGSLRLSCAASGyt-----vstYCMGWFRQ-APGK--EREGVATIL---G--GSTYYGDSVKGRFTISQDnAKNTVYLQMNSL-K-PEDTAIYYCAGSTVAstgwcsrlrpydYHYRGQGTQ-VTVS
1QD0  QVQLQE-SG---G-GLVQAGGSLRLSCAASGra--asghghYGMGWFRQ-VPGK--EREFVAAIRwSG--KETWYKDSVKGRFTISRDnAKTVYLQMNSL-K-GEDTAVYYCAARPVNvadis---lpvgEDYMGQGTQ-VTVS

1F2X  QVQLVE-SG---G-GSVQAGGSLRLSCAASGYt-----vSTYCMGWFRQ-APGK--EREGVATIL---G--GSTYYGDSVKGRFTISQDNAKNTVYLQMNSL-K-PEDTAIYYCAGSTVAstgwcstlrpyDYHYRGQGTQ-VTVS
8FAB  AVKLVQ-AG---G-GVVQPGRSLRLSCIASGFt-----fSNYGMHKVRQ-APGK--GLEWVAVIW--YngSRTYYGDSVKGRFTISRDNSKRTLYMQNSL-R-TEDTAVYYCARDPDlt------afSFDVWGQGVL-VTVS

1F2X  qVQLVE-SG---G-GSVQAGGSLRLSCAASGYt-----vstYCYCMGWFRQ-appk--eregvatil---g--gstyygdsvkgRFTISQDNakNTVYLQMNSL-K-pedtaiyyCAGStvastgwcsrlrpydyhyrgqgtq-vtvs
1VSC  -FKIET-TPesR-YLAQIGDSVSLTCSTTGce-------SPEFSMRTQ-id-----------spLNGKVTMEG--TTSTLTMNPV-S-FGNEHSYLCTATce------------srKLEK-GIQ-VEIY

1F2X  qvqlVE-SG---g-gsvgaggsLRLSCAAsgyt------vstYCMGWFRQ-apqk--erEGVATIl---g--gstyygdsvkgftISQDnAKNtVYLgmnsl-k-pedtaiyycagstvastgwcsrlrpydyhyrgqgtq-vtvs
1NS3  tqsfLA-TC---v-------nGVCNTVyhg--------agskTLAGP-k-------GPITq-----------MYTNVDQDLVGwgappg-a-rsltpctcgssdlylvethadvipvrrqdsrgsll-sprp

1F2X  QVQLVE-sg---g-GSVQAGGSLRLSCAASGYT-----vstYCMGWFRQ-APGK--EREGVATIl---g--gstYYGDSVKGRFTISOdnaknVYLOMNSL-k-PEDTAIYYCAGSTVAstgwcsrlrpydyhyrGQGTQ-VTVS
1F97  KGSVYT-aqs-d-VQVPENESIKLTCTYSGFS-------SPRVEWKFV-Q-GS--TIALVCYNS-------QITAPYADRVTFSS----SGITFSSV-T-RKDNGEYTCMVSEEgq--------DYGEVSIH-LTVL

1F2X  qVQLVE-SG---G-GSVQaggSLRLSCAASGvt-----vstYCMGWFRQ-APGK--EREGVATIl---g--gstYYgdsvkgftisqdnaknTVYLQMNSL-k-PedTAIYYCAGSTVAtgwcsrlrpydYHYRGQGTQ-VTVS
1FNF  vPPPTO-LR--FtNIGP--DTMRVTNAPPPs--------idLTNFLVRYSpVRNE--EDVAELSIS------------PSDNAVVLTNL-l-PgtEYVVSVSSVYEQh-----J------ESTPL-RGR-QKTG

1F2X  QVQLVE-SG---G-GSVgaggsLRLSCAASGyt-----vstYCMGNFRQ-APGK--EREGVATIl---g--gstYYgdsvkgftisqdnaknTVYLQMnsl-k-pedTAIYYCAGSTVastgwcsrlrpydYHYRGQGTQ-VTVS
1CEB  pNAPKLtGl--T-CQAd---xAEIHWEQQGdn-----rspiLHYTIQFN-TSFrpaSMDAYEKv----------pnTDSSFVVgms---pwanYTERVIAFNx----------igASPPSAHSDsCTTQ

1F2X  qvqLVE-SG---g-gsvgaGGSLRLSCAASGyt-----vstYCMGNFRQ-APGK--EREGVATIl---g--gstyygdsvkgrftisqdnaknVYLQMnsl-k-pedTAIYYCAGSTVastgwcsrlrpydyHYRGQGTQ-VTVS
1IAR  EapGNL-rvht----nvsOTLLLTWSNPYppdnylynHLTYAVNIsE-NDPA--DERIYNVTy-----------lePSLRIAas-tlksgiSYRARVRAWAQayn-------ttwSENSPSTKW-Hns

1F2X  qVQLVE-SG---g-gsvgaggslRLSCAASGvt-----vsTYCMGNFRQ-APgk--erEGVATIl---g--gstyygdsvkgftisqdnaknTVYLQMnsl-k-pedTAIYYCAGSTVastgwcsrlrpydyhyrgqgtq-VTVS
1F4H  tfQFRL-SG---------QTlEVTSey-lfrhsdNELLHMKVA-LDg---kPLASGEVP---ld-v-------apqqKQLIELPElpgpe-sagqLWLTVRVVQPnatav---seaghiSAWQ-QKR-LAEN
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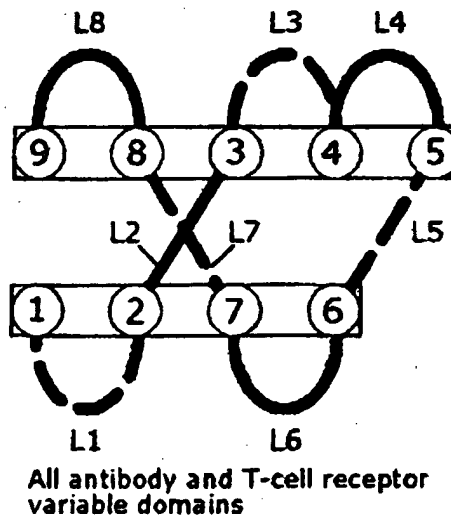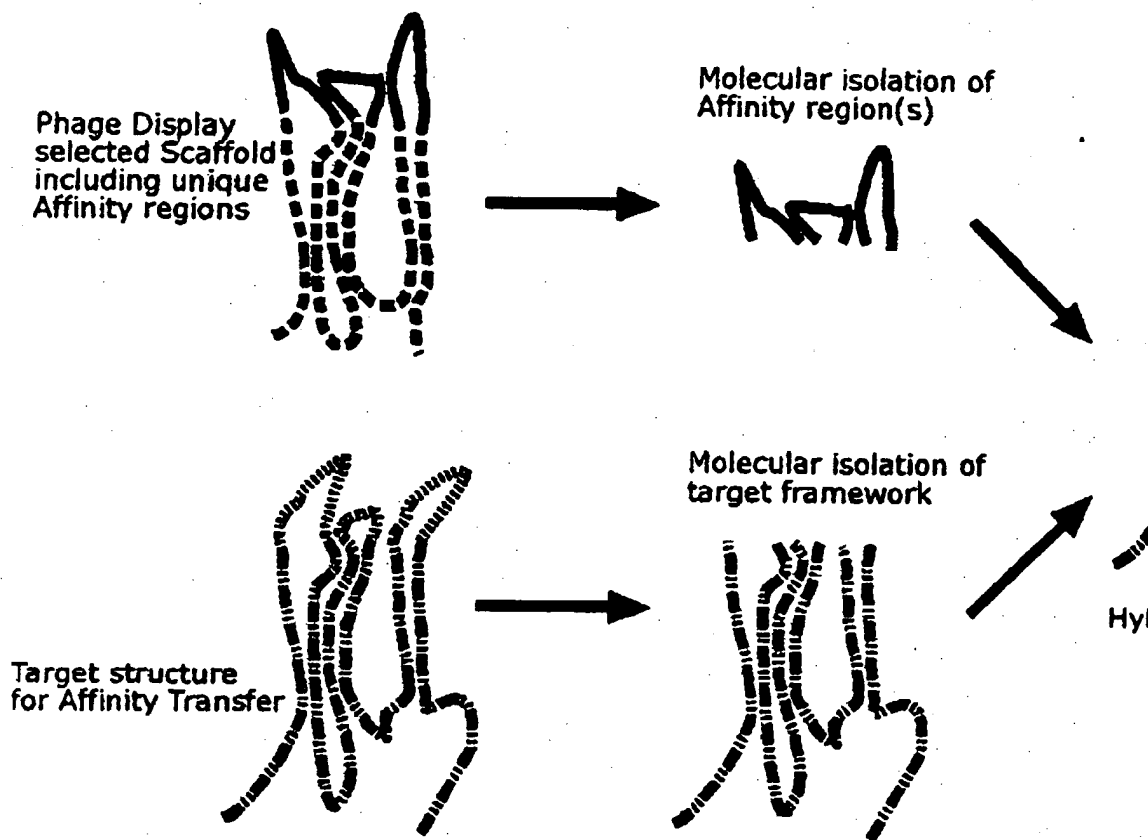
Figure elements explaned:

Underlined domains represent (putative) beta-elements 1-9
Capital letters indicate conserved structural amino acids
- indicate the absence of amino acid residues

Aligned protein domains from:

1F2X Single domain camelid antibody Cab-Ca05
1QD0 Camelid heavy chain variable domain
8FAB Heavy chain from human Igg1
1VSC Human Vcam-1
1NS3 Structure Of Hcv Protease (Bk Strain) from hepatitis C-virus
1F97 Soluble Part Of The Junction Adhesion Molecule From Mouse
1FNF Fragment Of Human Fibronectin Encompassing Type-Iii Repeats 7 Through 10
1CFB Drosophila neuroglian
1IAR Human Interleukin-4 receptor alpha chain complex
1F4H E. Coli (Lacz) Beta-Galactosidase (Orthorhombic)

Fig. 5

Scaffold with V$_{HH}$ 1MEL CDR regi ns

```
 N  V  K  L  V  E  K  G  G  N  F  V  E  N  D  D  D  L  K  L
AATGTGAAACTGGTTGAAAAAGGTGGCAATTTCGTCGAAAACGATGACGATCTTAAGCTC
 T  C  R  A  E  G  Y  T  I  G  P  Y  C  M  G  W  F  R  Q  A
ACGTGCCGTGCTGAAGGTTACACCATTGGCCCGTACTGCATGGGTTGGTTCCGTCAGGCG
 P  N  D  D  S  T  N  V  A  T  I  N  M  G  G  G  I  T  Y  Y
CCGAACGACGACAGTACTAACGTGGCCACGATCAACATGGGTGGCGGTATTACGTACTAC
 G  D  S  V  K  E  R  F  D  I  R  R  D  N  A  S  N  T  V  T
GGTGACTCCGTCAAAGAGCGCTTCGATATCCGTCGCGACAACGCGTCCAACACCGTTACC
 L  S  M  D  D  L  Q  P  E  D  S  A  E  Y  N  C  A  G  D  S
TTATCGATGGACGATCTGCAACCGGAAGACTCTGCAGAATACAATTGTGCAGGTGATTCT
 T  I  Y  A  S  Y  Y  E  C  G  H  G  L  S  T  G  G  Y  G  Y
ACCATTTACGCGAGCTATTATGAATGTGGTCATGGCCTGAGTACCGGCGGTTACGGCTAC
 D  S  H  Y  R  G  Q  G  T  D  V  T  V  S  S
GATAGCCACTACCGTGGTCAGGGTACCGACGTTACCGTCTCGTCG
```

Scaffold with V$_{HH}$ 1BZQ CDR regions

```
 N  V  K  L  V  E  K  G  G  N  F  V  E  N  D  D  D  L  K  L
AATGTGAAACTGGTTGAAAAAGGTGGCAATTTCGTCGAAAACGATGACGATCTTAAGCTC
 T  C  R  A  S  G  Y  A  Y  T  Y  I  Y  M  G  W  F  R  Q  A
ACGTGCCGTGCTAGCGGTTACGCCTACACGTATATCTACATGGGTTGGTTCCGTCAGGCG
 P  N  D  D  S  T  N  V  A  T  I  D  S  G  G  G  G  T  L  Y
CCGAACGACGACAGTACTAACGTGGCCACCATCGACTCGGGTGGCGGCGGTACCCTGTAC
 G  D  S  V  K  E  R  F  D  I  R  R  D  K  G  S  N  T  V  T
GGTGACTCCGTCAAAGAGCGCTTCGATATCCGTCGCGACAAAGGCTCCAACACCGTTACC
 L  S  M  D  D  L  Q  P  E  D  S  /A  E  Y  N  C  A  A  G  G
TTATCGATGGACGATCTGCAACCGGAAGACTCTGCAGAATACAATTGTGCAGCGGGTGGC
 Y  E  L  R  D  R  T  Y  G  Q  R  G  Q  G  T  D  V  T  V  S
TACGAACTGCGCGACCGCACCTACGGTCAGCGTGGTCAGGGTACCGACGTTACCGTCTCG
 S
TCG
```

Scaffold with V$_{HH}$ 1HCV CDR regions

```
 N  V  K  L  V  E  K  G  G  N  F  V  E  N  D  D  D  L  K  L
AATGTGAAACTGGTTGAAAAAGGTGGCAATTTCGTCGAAAACGATGACGATCTTAAGCTC
 T  C  R  A  E  G  R  T  G  S  T  Y  D  M  G  W  F  R  Q  A
ACGTGCCGTGCTGAAGGTCGTACGGGTTCGACCTACGATATGGGTTGGTTCCGTCAGGCG
 P  N  D  D  S  T  N  V  A  T  I  N  W  D  S  A  R  T  Y  Y
CCGAACGACGACAGTACTAACGTGGCCACGATCAACTGGGATAGCGCCCGTACGTACTAC
 G  D  S  V  K  E  R  F  D  I  R  R  D  N  A  S  N  T  V  T
GGTGACTCCGTCAAAGAGCGCTTCGATATCCGTCGCGACAATGCCTCCAACACCGTTACC
 L  S  M  D  D  L  Q  P  E  D  S  A  E  Y  N  C  A  G  G  E
TTATCGATGGACGATCTGCAACCGGAAGACTCTGCAGAATACAATTGTGCAGGTGGTGAA
 G  G  T  W  D  S  R  G  Q  G  T  D  V  T  V  S  S
GGCGGCACCTGGGATAGCCGTGGTCAGGGTACCGACGTTACCGTCTCGTCG
```

Underlined regions indicate specific affinity regions.
The sequence of underlined regions in each panel represent respectively loop L2
(~CDR1 and AR1), L4 (~CDR2 and AR2) and L8 (~CDR3 and AR4).

Fig. 6a

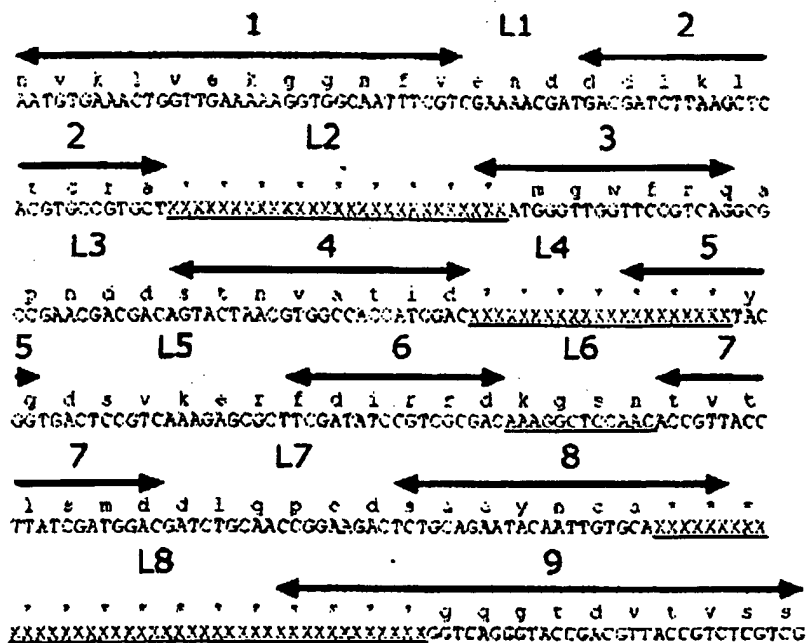## Structural topology of a primary scaffold

Fig. 6b